

SUPPORTING INFORMATION

(8 pages containing 7 figures)

Charcoal disrupts soil microbial communication through a combination of signal sorption and hydrolysis

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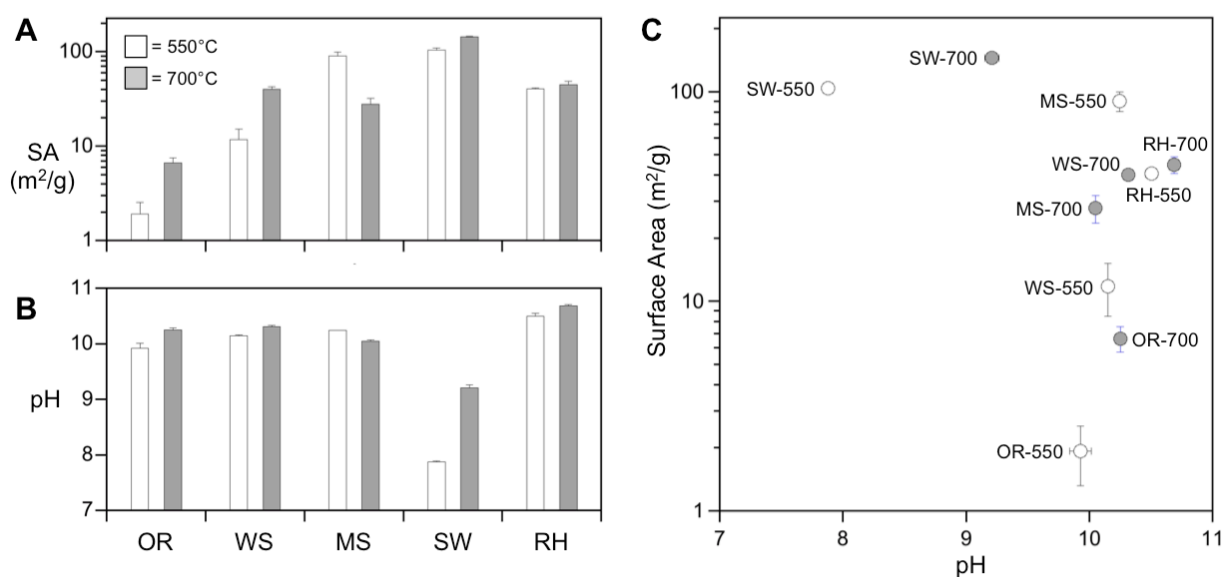


Figure S1. Physical and chemical characterization of the UKBRC charcoals. (A) N₂ sorption analysis was applied to charcoals generated at 550°C and 700°C, and surface area was calculated using BET. The charcoals were prepared from oilseed rape (OR), wheat straw (WS), miscanthus straw (MS), soft wood (SW), and rice husk (RH) feedstocks. (B) The pH of 1 mL solutions reacted with 50 mg charcoal (a 1 to 20 solid/liquid ratio) for 1.5 hours at 25°C. (C) Comparison of charcoal surface area and pH reveal that soft wood displays the highest surface area and lowest pH values, while oilseed displays the lowest surface area and higher pH values. All measurements were performed in triplicate and are reported as the mean \pm 1 standard deviation.

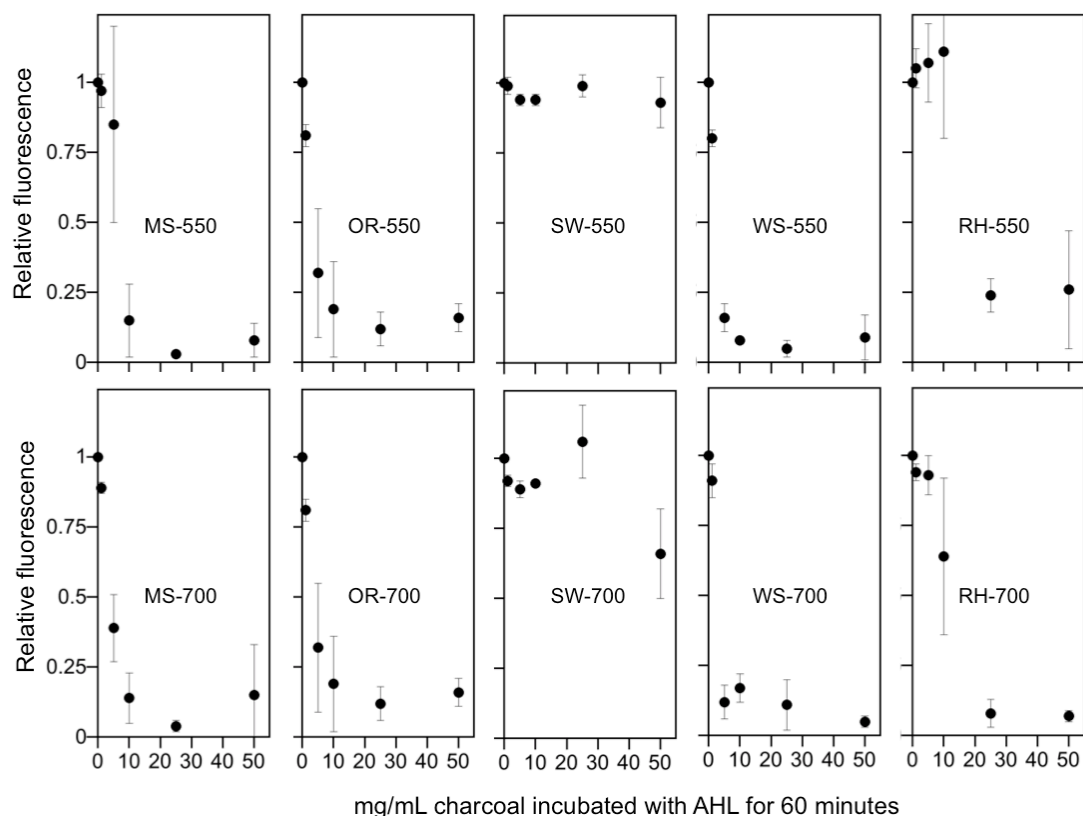


Figure S2. Effect of charcoal concentration on AHL-dependent GFP expression within *E. coli*. Varying concentrations of each charcoal were reacted with AHL for 1 hour, charcoals were pelleted by centrifugation, and the soluble fractions were added to cells programmed to produce GFP upon exposure to AHL. After overnight growth, AHL-induced GFP expression was quantified by measuring green fluorescence and normalizing the whole cell fluorescence signal to the cell density. The relative fluorescence values in the presence of charcoals were scaled against the signal obtained with untreated AHL. All measurements were performed in triplicate and are reported as the mean $\pm 1\sigma$.

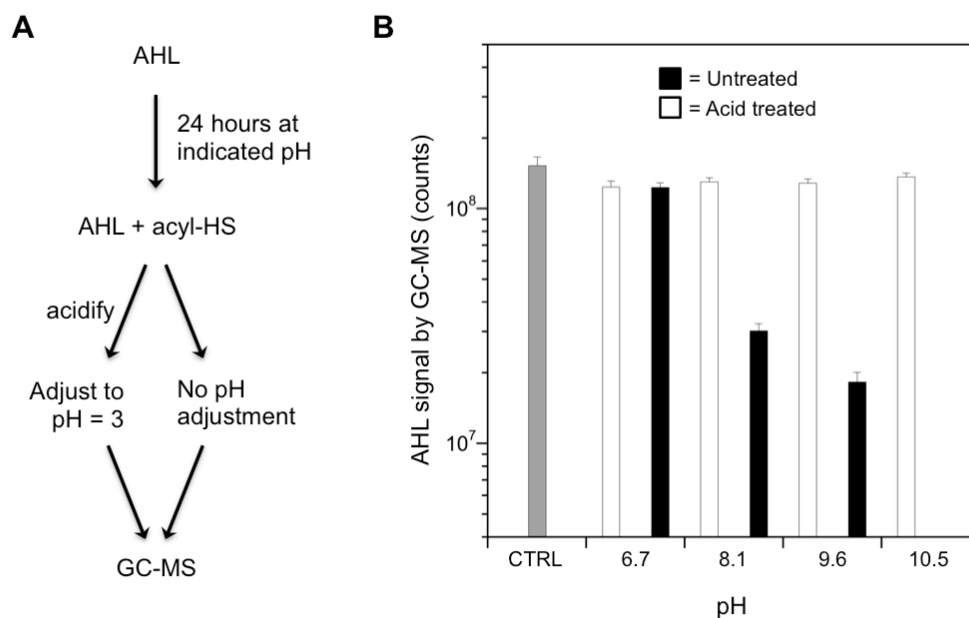


Figure S3. Recovery of AHL using acidification. (A) Solution containing 50 μ M AHL were adjusted to different pH values (6.7, 8.1, 9.6, 10.5), and reacted for 24 hours. At the end of the reaction, samples were split into two fractions, one fraction was adjusted to pH 3 while the other fraction was left untreated. (B) GC-MS signal obtained from acidified and untreated samples. As a control AHL was reacted in a solution at a low pH that favors conversion of Acyl-HS into AHL. Error bars represent $\pm 1\sigma$ calculated using three independent measurements.

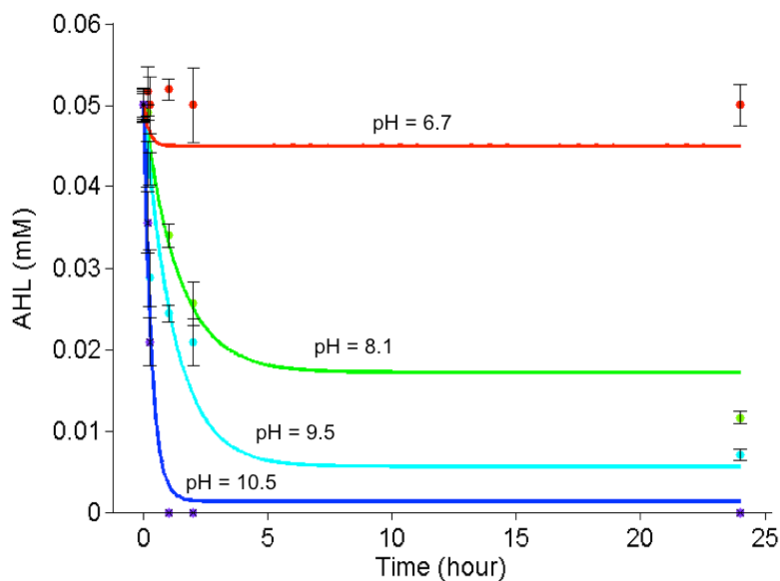


Figure S4. Effect of pH on AHL hydrolysis kinetics. Solution containing 50 μM AHL were reacted for different lengths of time (0.016, 0.25, 1, 2, and 24 hours) at four different pH conditions (6.7, 8.1, 9.6, 10.5). The lines represent a global fit of all kinetic data simultaneously to the ODE model that considers the two different mechanisms of hydrolysis, which yielded rate constants for pH-dependent ($k_{\text{hyd1}} = 8.36 \text{ mM}^{-1}\text{hr}^{-1}$; $k_{\text{dehyd2}} = 2.03 \times 10^4 \text{ mM}^{-1}\text{hr}^{-1}$) and pH-independent ($k_{\text{hyd1}} = 0.46 \text{ hr}^{-1}$; $k_{\text{dehyd2}} = 0.085 \text{ hr}^{-1}$) reactions.

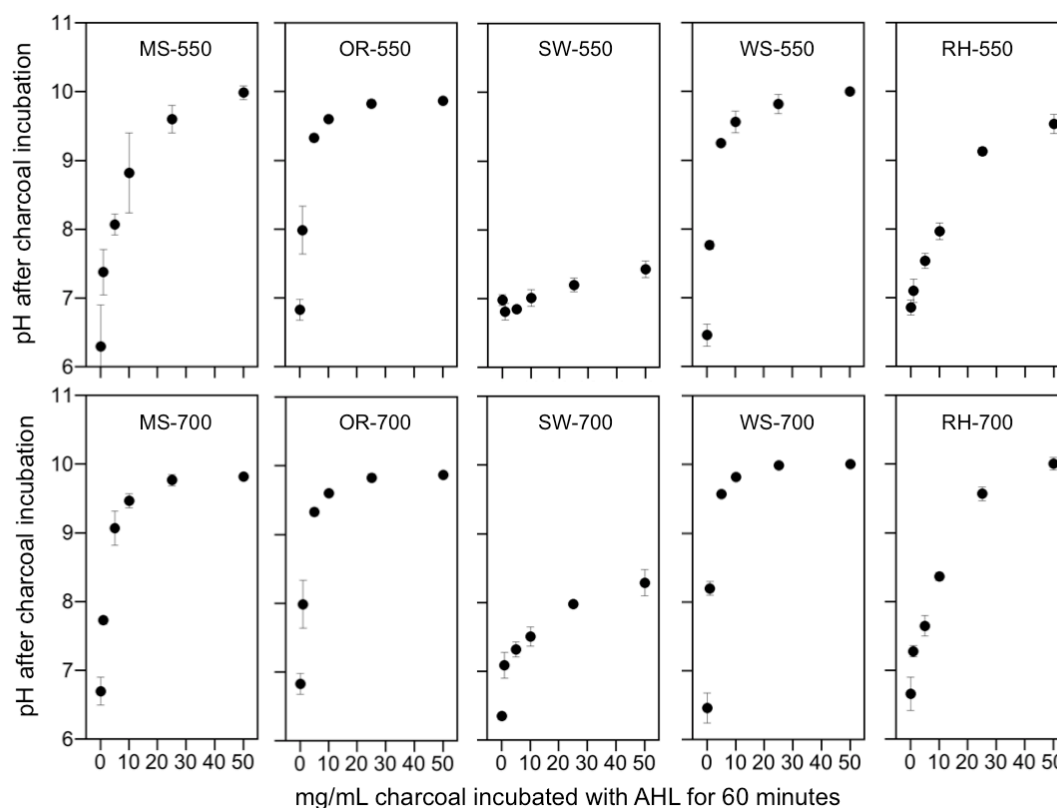


Figure S5. Relationship between charcoal concentration and charcoal-induced pH changes. For each of the experiments shown in Figure S2, we measured the solution pH at the end of reactions to establish how increasing concentrations of each charcoal influence solution pH. All measurements were performed in triplicate and are reported as the mean $\pm 1\sigma$.

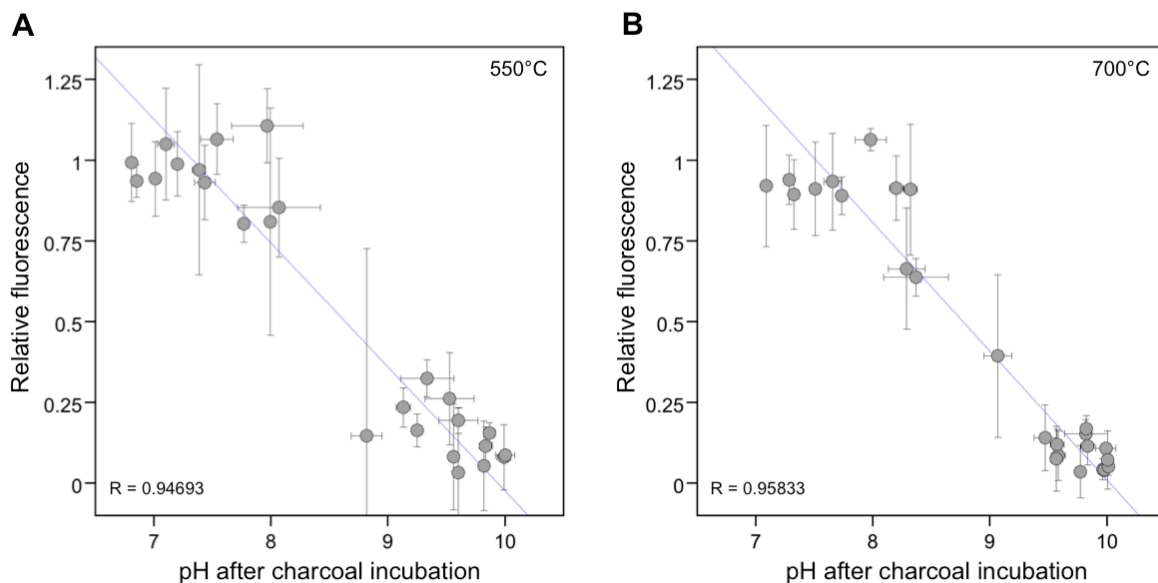


Figure S6. Relationships between charcoal alkalinity and AHL-dependent GFP expression. The relative fluorescence of *E. coli* mixed with charcoal-treated AHL is compared with the pH of each AHL-containing solution after reaction for 1 hour with charcoals generated through pyrolysis at (A) 550°C and (B) 700°C. Linear fits to the 550°C (Relative fluorescence = $3.4688 - 0.34357\text{pH}$) and 700° (Relative fluorescence = $3.7001 - 0.36549\text{pH}$) charcoal data yield $R = 0.947$ and 0.958 , respectively.

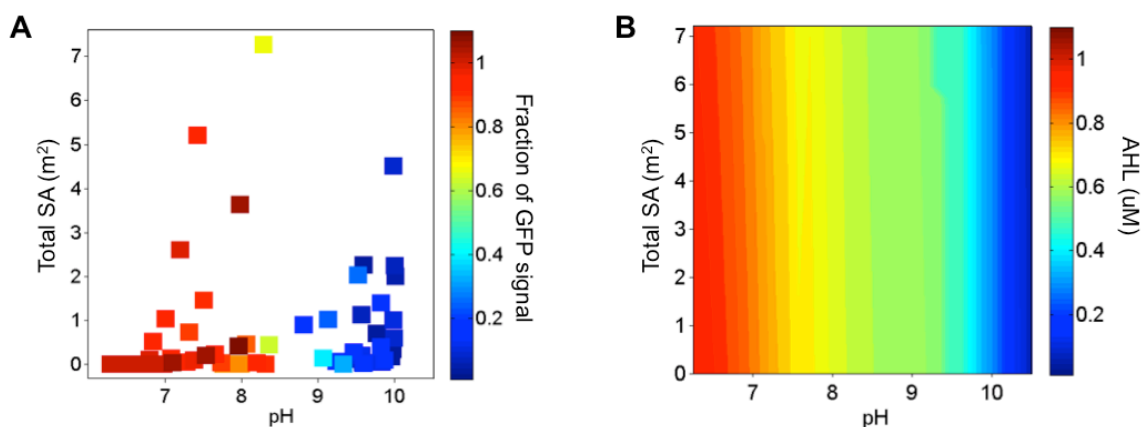


Figure S7. A kinetic model captured the concentration change of AHL after reaction with the UKBRC charcoals for 1 hour. (A) The microbial assay results described in Figure S2 were plotted as a function of pH and SA to illustrate the fraction of AHL-dependent GFP signal remaining after incubation of different concentrations of each charcoal with AHL. Total SA was calculated as the product of the mass of a charcoal added in grams (g) and the value obtained from BET analysis (m^2/g). **(B)** A kinetic model was used to calculate the concentration of AHL that remains biologically available after 1 hour reaction with charcoals varying in a range of pH and SA properties. The color gradient indicates the concentration of biologically available AHL at the end of the reaction.